Biochemical aspects of symbiosis between termites and their intestinal microbiota

JOHN A. BREZNAK

Department of Microbiology and Public Health, Michigan State University, East Lansing, Michigan 48824-1101, USA

Key words: termites; invertebrate gut microbiota; enzyme activity; nitrogen metabolism; wood digestion

Introduction

A fascinating and frequently cited example of nutritional symbiosis is the association between certain xylophagous termites and their intestinal protozoa. The classical early studies by Cleveland (1923, 1924, 1925*a*, *b*), Trager (1932, 1934), and Hungate (1938, 1939, 1943) indicated that the protozoa were key cellulolytic agents in certain groups and that their presence in the gut was critical to the survival of termites on a diet of sound wood or cellulose. However, this is an oversimplification of the biochemical details of the symbiosis, since only fragmentary information has been available on the importance of the bacterial component of the termite's intestinal microbiota. Further, most extant termite species harbour only bacteria in their intestinal tract.

Several factors have undoubtedly contributed to the slow pace at which research in this area has progressed. First, the relatively small size of most termites hampers *in situ* studies of the gut microbiota, as well as biochemical studies on termite tissues. Second, the diversity and complexity of the gut microbiota (discussed below) makes the microbial community inherently difficult to analyse from a functional standpoint. Third, the refractility of many of the gut organisms to *in vitro* cultivation, coupled with the lack of an available system to rear germ-free termites, has made it difficult to determine the quantitative importance of individual gut microbes, versus the biochemical capabilities of the insect, in meeting the termite's nutritional requirements. Nevertheless, some of these difficulties have been overcome in recent years, so that we now have a better understanding of the role of gut microbes in termite digestive processes and nutrition. Accordingly, the purpose of this review is to summarize our current knowledge on this topic with particular reference to research conducted in the last decade.

In this review the term symbiosis, and derivatives thereof, will be used in the broad sense of de Bary (1879), i.e. the permanent or semi-permanent association of two dissimilar organisms (symbionts). More specific terms will be used where appropriate and where possible. For other perspectives on this topic, as well as more detailed information on termite nutrition, behaviour, and ecology, several additional reviews are available (Breznak, 1975, 1982; Brian, 1978; Hungate, 1946*a*; Krishna & Weesner, 1969–1970; LaFage & Nutting, 1978; Lee & Wood, 1971; O'Brien & Slaytor, 1982; Potrikus, 1983).

Nutritional biology of termites

Of all the intriguing activities and properties of termites, none seems as widely recognized (or as often quoted) as the ability of termites to utilize wood as a food resource. Indeed, many species of termites thrive on sound, decay-free wood which contains as little as 0.03–0.1% nitrogen (dry weight basis; Cowling & Merrill, 1966). However, it is also important to recognize that there are nearly 2000 species of termites, and their biology and behaviour can be quite varied (Krishna, 1969). For example many species prefer wood that is partially decayed by fungi (Lee & Wood, 1971), whereas some termites (subfamily Macrotermitinae) actually cultivate fungi in elaborate gardens for use as a nutrient resource (Sands, 1969). Still others, depending on the species, feed on leaves, roots, grasses, dung of herbivorous animals, humus, or soil (Lee & Wood, 1971). Clearly, the diet of termites as a group is quite diverse, but is basically one rich in cellulose, hemicellulose, and lignin or lignin derivatives. Since their diet is also relatively poor in combined nitrogen, termites may be thought of as oligonitrotrophic saprovores. This trait places termites in an important position ecologically, particularly in tropical regions where their activities can dominate the processes of decomposition and nutrient cycling. Further, the biomass density of termites can be so large (10–20 $\mathrm{g\,m^{-2}}$) that their impact is similar to, and may surpass, that of grazing mammals (Wood & Sands, 1978).

The termite gut as a microbial habitat

All termites that have been examined possess a dense and diverse population of microorganisms in their alimentary tract. In the phylogentically 'lower' termites (families Masto-, Kalo-, Hodo-, Rhino-, and Serritermitidae) the intestinal microbiota includes bacteria, as well as unique genera and species of oxymonad, trichomonad, and hypermastigote protozoa found almost nowhere else in nature (Honigberg, 1970; Yamin, 1979). Many of these protozoa are capable of ingesting wood particles and are cellulolytic. In the phylogenetically 'higher' termites (family Termitidae), which constitute roughly 75% of all termite species, the gut microbiota consists essentially of bacteria alone. In those instances where protozoa have been observed in higher termites, their populations were generally low and the protozoa were not of the cellulolytic type (Honigberg, 1970). At present, there is no evidence to indicate that any termite harbours a prominent and stable population of fungi in the gut.

Anatomy

The gross anatomy, histology, and cytology of the termite alimentary tract, as well as its phylogenetic variations, have been described in detail by Noirot & Noirot-Timothée (1969). Basically, the termite gut consists of three main divisions: the foregut or stomodeum (which includes the crop and muscular gizzard); the tubular midgut or mesenteron; and the voluminous hindgut or proctodeum (which includes the paunch, colon and rectum). For the present discussion it is the bulbous 'paunch' region of the hindgut which is particularly important, because it is almost invariably colonized by a dense microbiota. The paunch region of the gut of a lower termite, *Reticulitermes flavipes*, is shown in Fig. 7.1. The digestive tube of higher termites shows greater anatomical variability and may also include a mixed segment. The latter is formed by a posterior elongation of part of the mesenteron, such that the lumen of the digestive tube is bounded on one side by the mesenteron and on the other side by the proctodeum.

Studies of intestinal transit of food have been few, but indicate that passage of food through the gut takes about 24 hours with the food being retained longest in the hindgut (Kovoor, 1967*a*; Noirot & Noirot-Timothée, 1969). Recent work by Odelson and Breznak (unpublished), who tracked food movement through the gut of *Reticulitermes flavipes* by using sawdust amended with ^{14}C-labelled lignin, showed that the retention time of particulate food in the hindgut was 26 hours.

An enteric valve prevents refluxing of hindgut contents to the midgut, which is the main site of nutrient absorption in most insects. Consequently, microbial fermentation products, which are produced primarily in the hindgut, are absorbed from the hindgut. Unless proctodeal

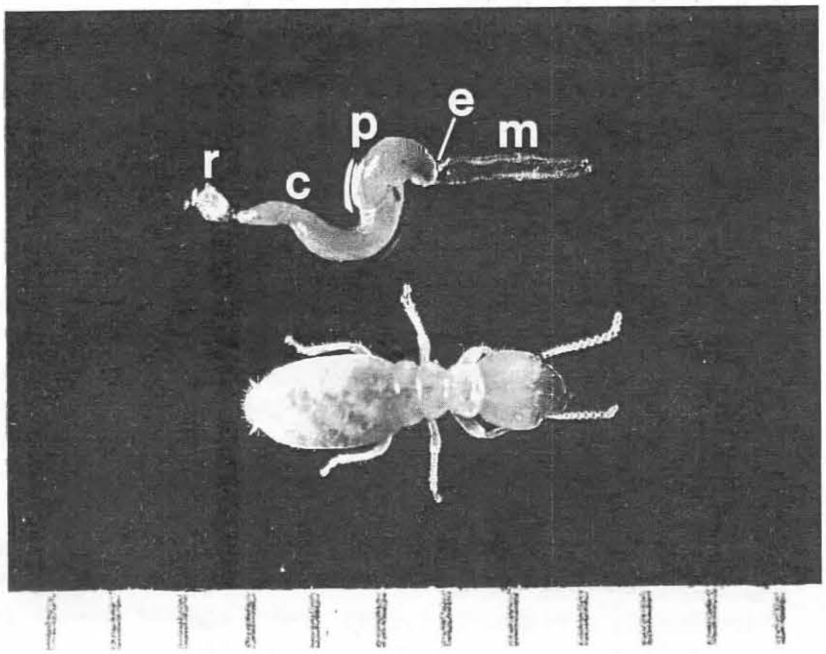

Fig. 7.1. A gut from a *Reticulitermes flavipes* worker (top), extracted from the posterior end of the insect with fine-tipped forceps, is positioned next to a separate, intact worker. Note the bulbous paunch region of the hindgut. Malpighian tubules are not readily seen in this preparation, because they are very closely apposed to the hindgut. Individual marks on the rule are 1 mm apart. Abbreviations: c, colon; e, enteric valve region; m, midgut; p, paunch; r, rectum.

trophallaxis occurs, absorption in the midgut is limited to soluble nutrients present in the food, or those liberated from the food by termite-secreted enzymes or by bacteria which may colonize the midgut (see below).

Another key feature of the alimentary tract is the presence of Malpighian tubules which empty at the precise juncture of the midgut and hindgut. Consequently, urine and urinary metabolites must traverse the hindgut before either being voided to the exterior with faeces, or resorbed from the hindgut, or metabolized by hindgut microorganisms.

Physico-chemical characteristics of the gut

The physico-chemical characteristics of the gut are similar in both higher and lower termites (reviewed by O'Brien & Slaytor, 1982).

The pH in regions where microbes are found (hindgut and midgut) usually ranges from about 6.0 to 7.5. Exceptions exist, however, in the mixed segment of higher termites (pH 7.0 to >9.6), as well as in the anterior hindgut of some soil-feeding termites where pH values as high as 10.4 have been recorded (Bignell & Anderson, 1980). In addition, sites such as the paunch which are heavily colonized by microorganisms appear anaerobic with an E_0' usually in the range of -230 to $-270\,\mathrm{mV}$. By contrast, midgut regions appear to be aerobic. Such determinations have been made by feeding the insects redox dyes and observing the resulting colour in the gut (Veivers, O'Brien & Slaytor, 1980), or by physiological measurements on gut homogenates (Bignell & Anderson, 1980). Anaerobicity of the hindgut is consistent with the oxygen sensitivity of the hindgut protozoa of lower termites (Cleveland, 1925*a*; Hungate, 1939), with the demonstration of strict anaerobic bacteria in termite hindguts (Schultz & Breznak, 1978; Potrikus & Breznak, 1980*b*), with the demonstration of *in situ* methanogenesis by the hindgut microbiota (Breznak, 1975), and with the isolation of methanogenic bacteria from homogenates of termite hindguts (Odelson and Breznak, unpublished).

Gut microbes and sites of colonization

As mentioned, the hindgut is heavily colonized by microorganisms. This is true for both lower and higher termites. By using light and electron microscopy, Breznak (1975) and Breznak & Pankratz (1977) found the hindgut of *Reticulitermes flavipes* and *Coptotermes formosanus* to be colonized by a morphologically heterogeneous assemblage of bacteria and protozoa. Most of the bacteria were situated close to the paunch epithelium, and many possessed holdfast elements that appeared to secure them to the epithelial tissue and to other bacterial cells. Lesser numbers of bacteria were observed free in the lumen of the paunch, which was occupied mainly by protozoa. However, some of the protozoa (*Pyrsonympha vertens*) also possessed a holdfast organelle that enabled them to attach by their anterior end to the paunch epithelium. The midgut was more sparsely colonized, but did possess distinctive cuboidal-shaped, endospore forming bacteria situated between the microvilli of the epithelium. Similar observations have been made with other lower (Bloodgood, 1975; To, Margulis, Chase & Nutting, 1980) and higher termites (Kovoor, 1959, 1968; Noirot & Noirot-Timothée, 1967). In the soil-feeding higher termites *Procubitermes aburiensis* and *Cubitermes severus*, the proctodeum is heavily colonized by bacteria, and many of these bacteria are attached to cuticular spines emanating from the wall of the posterior colon (Bignell, Oskarsson & Anderson, 1979, 1980*a*, *b*). Some of the bacteria are filamentous, actinomycete-like forms. The crop, mesenteron, and mixed segment also harboured bacteria (Bignell *et al.*, 1980*c*). Interestingly, when a mixed segment is present the bacteria therein tend to be so similar in morphology as to suggest a pure culture (Noirot & Noirot-Timothée, 1969).

As determined by direct microscopic count, population levels of bacteria range from 10^6–10^7 bacteria gut^{-1} (Krasil'nikov & Satdykov, 1969; Breznak, Brill, Mertins, and Coppel, 1973; Schultz & Breznak, 1978), whereas those of protozoa are about 3–4×10^4 gut^{-1} for *Reticulitermes flavipes* (Mauldin & Rich, 1980). Considering that the gut of some termites such as *R. flavipes* is 1 μl or less in volume, these data translate to about 10^9–10^{10} bacteria ml^{-1} and 10^7 protozoa ml^{-1} gut contents, i.e. the gut (particularly the hindgut) is almost a solid, packed mass of microbes. Indeed, Odelson & Breznak (1983) determined that 61% of the hindgut contents of *R. flavipes* was microbial cells, the remainder being extracellular hindgut fluid. It is noteworthy that the ratio of protozoan cells to bacteria in the hindgut of lower termites such as *R. flavipes* (1:100) is quite large compared to a gastrointestinal ecosystem such as the bovine rumen (1:100 000; Hungate, 1975). This undoubtedly has a bearing on the pattern of fermentation in the hindgut and is discussed further below.

Attempts at enumeration and identification of termite gut bacteria by means of microbiological culture techniques have been reviewed by Breznak (1975, 1982) and O'Brien & Slaytor (1982). Most of the bacteria isolated from lower and higher termites have proven to be facultative or strict anaerobes including strains of *Streptococcus*, *Bacteroides*, various Enterobacteriaceae, *Staphylococcus*, and *Bacillus*. The biochemical characteristics of these bacteria, as they relate to symbiotic interaction with the termites, are discussed in the appropriate sections below.

Spirochaetes are of constant and abundant occurrence in the hindgut of all termites examined and exhibit considerable diversity in terms of their size range (0.2×3.0 to 1.0×100 μm) and morphology (Breznak, 1973, 1983; To *et al.*, 1980). They generally occur free in the gut fluid, as well as attached to the surface of polymastigote and hypermastigote protozoa in hindguts of lower termites (Breznak, 1983). In some of the latter associations they have evolved spectacular 'motility symbioses', whereby their coordinated undulations can actually serve to propel the protozoans through the hindgut fluid. This has been described for spirochaetes adherent to *Mixotricha paradoxa* from the hindgut of the primitive Australian termite *Mastotermes darwiniensis* (Cleveland & Grimstone, 1964), as well as for spirochaetes adherent to unidentified flagellates from *Kalotermes schwarzi, Pterotermes occidentis*, and *Marginitermes hubbardi* (To, Margulis & Cheung, 1978). Unfortunately, almost nothing is known of their biochemical role in the gut system, as termite hindgut spirochaetes have defied all attempts at *in vitro* cultivation (Breznak, 1973). However, they may be important to the vitality of termites. Eutick, Veivers, O'Brien & Slaytor (1978) observed decreased survival of the higher termite *Nasutitermes exitiosus* when spirochaetes were eliminated from the gut by means of antibiotics.

Digestive processes in termites and the role of gut microorganisms

Cellulose and hemicelluloses (xylans, mannans, etc.) undergo substantial dissimilation on passage through the termite gut (65–99%), and the assimilation efficiency of wood-feeders is quite high (54–93%) (Esenther & Kirk, 1974; Wood, 1978). Values for lignin digestion vary considerably and will be discussed later. Nevertheless, a key question has been, and continues to be, the contribution of specific gut organisms, versus the termite's own enzymes, to the digestive process.

Lower termites

The bulk of wood polysaccharide digestion occurs in the hindgut of lower termites, and all available evidence indicates that the hindgut microbiota, principally the anaerobic flagellate protozoa, is the driving force of such dissimilatory activity (reviewed by Breznak, 1975, 1982 and O'Brien & Slaytor, 1982). The early work of Hungate (1938, 1939, 1943) in particular led to the following model for wood-cellulose digestion. Wood particles consumed by termites are passed to the hindgut where they are endocytosed by protozoa. The cellulose is fermented anaerobically within the protozoa to CO_2, H_2 and acetate, which are liberated from the cells. Acetate is subsequently absorbed by the termites and used as their major oxidizable energy source. This model is conceptually appealing, because it permits acquisition of energy (ATP) by both the protozoa (via anaerobic fermentation of cellulose) and the termites (via aerobic oxidation of acetate).

Acetate is indeed the major volatile fatty acid (VFA) in hindgut fluid of lower termites examined. Odelson & Breznak (1983) used a 'micro'

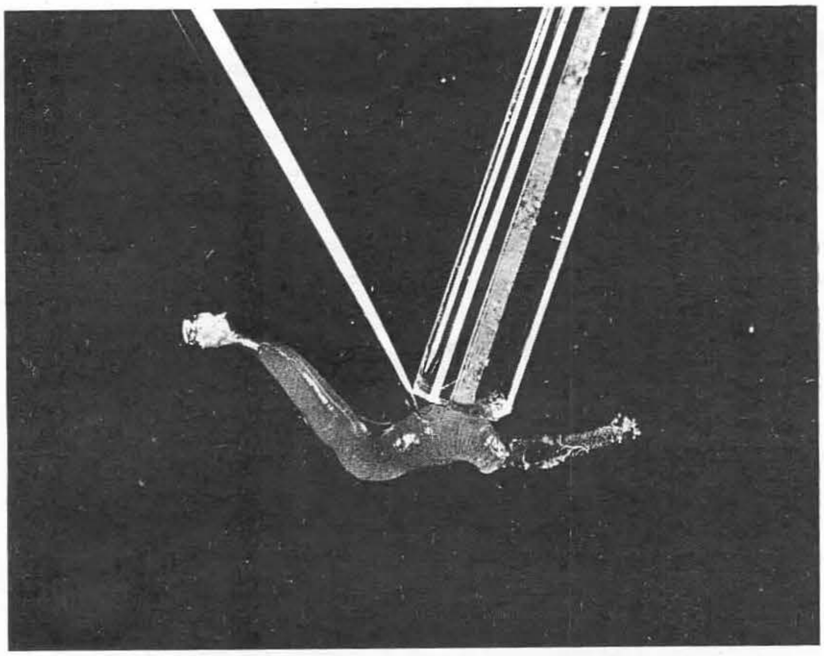

Fig. 7.2. A 'micro' sampling method is used to sample paunch contents from (in this case) the gut of *Reticulitermes flavipes*. After puncturing the paunch with a fine dissecting needle (left), the contents which issue from the puncture site are immediately aspirated into a 1 μl capacity glass capillary tube (right).

technique to sample hindgut fluid from termites for subsequent analysis by gas chromatography and mass spectroscopy (Fig. 7.2). These investigators found that acetate was present in extracellular hindgut fluid of worker termites (*Reticulitermes flavipes, Zootermopsis angusticollis*, and *Incistermes schwarzi*) at concentrations of 58–81 mM, and it accounted for 94–99 mole % of all VFAs. Small amounts of propionate and butyrate were also detected. By feeding *R. flavipes* sawdust amended with ^{14}C-labelled polymers, they also found that about 80% of the acetate was derived from cellulose, whereas about 20% was derived from hemicellulose.

If the main role of protozoa is to degrade wood-cellulose to acetate, one would predict that defaunated termites (i.e. with protozoa removed) should be able to survive on a cellulose-free diet if supplied with acetate. While this has been tried, negative results were obtained with

Z. angusticollis (Cook, 1943; Hungate, 1946*a*) and with *R. flavipes* (Odelson & Breznak, 1983). However, the lack of success might be due to toxicity to termites of the cation moiety (Ca^{2+} or Na^+) of the acetate salt that was added to the diet. It should be remembered that in the faunated termites there is a continuous and gradual production of free acetic acid – a situation difficult to mimic in feeding studies.

A major contribution to our understanding of termite hindgut protozoa is the recent work of Yamin (1978, 1981), who obtained axenic cultures of major cellulolytic species *Trichomitopsis termopsidis* and *Trichonympha sphaerica* from the termite *Zootermopsis*. The medium used to cultivate the protozoa was prepared under anaerobic conditions and included: cellulose, serum, yeast extract, glutathione, and dead (heat-killed) rumen bacteria. Initial cultures also contained antibiotics to inhibit bacterial growth, and final axenic cultures appeared to be free of live extracellular bacteria as well as endosymbiotic bacteria. Both species of protozoa were cellulolytic and fermented cellulose to CO_2, H_2, and acetate as major products (Yamin, 1980, 1981). These products were identical to those formed from cellulose by mixed suspensions of hindgut protozoa from *Zootermopsis* (Hungate 1939, 1943). For *T. termopsidis*, cellulose in the culture medium could not be replaced by glucose, cellobiose, carboxymethylcellulose, glycogen, inulin, or rice starch (Yamin, 1978). Moreover, the dead rumen bacteria (which were endocytosed and digested within food vacuoles) could not be replaced by cells of *Escherichia coli, Bacillus subtilis, Pseudomonas maltophilia*, or a *Clostridium* sp. Presumably, the rumen bacteria were the *in vitro* correlate of termite hindgut bacteria (on which the protozoa graze *in vivo*) and one or more species in the mixture provided a growth factor necessary for the protozoa. Crude extracts of *T. termopsidis* possessed carboxymethylcellulase (Yamin & Trager, 1979), as well as cellobiase, coenzyme-A-dependent pyruvate : ferredoxin oxidoreductase, and hydrogenase (Yamin, 1980).

If defaunated *Zootermopsis* was reinfected with axenic cultures of *T. termopsidis*, the termites survived appreciably longer than did non-reinfected controls and as well as those reinfected with a mixed population of microbes derived from hindgut contents (Yamin & Trager, 1979).

These experiments buttressed the conclusion that hindgut protozoa are key agents of cellulose digestion in lower termites, and they showed that cellulase activity in the protozoa does not necessarily require the presence of endosymbiotic bacteria (Yamin, 1981). The availability of pure cultures should make these protozoa important subjects for further biochemical studies.

Considering the extent to which wood polysaccharides are dissimilated in termites, it appears that the protozoa are quite efficient at stripping these polymers from their complex with lignin. Accordingly, it would be of intrinsic interest, and perhaps also practical value, to study the nature of their cellulases and other carbohydrases in greater detail. Unfortunately, the growth yields of the protozoa *in vitro* are relatively poor. For example, cell yields of *T. termopsidis* are about 2000 cells ml^{-1} after 37 days (Yamin, 1980), whereas those of the larger *T. sphaerica* are only 150 cells ml^{-1} (Yamin, 1981). Accordingly, in order to facilitate biochemical studies, efforts were made in our laboratory to increase the cell yields of these protozoa. Since hydrogen was the only reduced product formed during growth of these protozoa, it seemed likely that some of the H_2 was derived from NADH + H^+. However, H_2 production from NADH + H^+ is thermodynamically unfavourable even at partial pressures of H_2 as low as 10^{-3} atmospheres (Wolin, 1974). This suggested that accumulation of H_2 in protozoan cultures might actually be suppressing cell yields. Accordingly, attempts were made to co-culture *T. termopsidis* with avid hydrogen-consuming bacteria. It was reasoned that if the pH_2 in protozoan cultures could be kept very low, by employing hydrogen-consuming bacteria as an 'electron sink' (Wolin, 1974), better growth of the protozoa might result. Preliminary experiments (Table 7.1; Odelson and Breznak, unpublished) revealed that yields of *T. termopsidis* were increased in the presence of a methane-forming *Methanospirillum*. Additionally, it was found that replacement of dead rumen bacteria in the culture medium with heat-killed cells of *Bacterioides* sp. strain JW20, a bacterium isolated from the hindgut of *Reticulitermes flavipes* (Schultz & Breznak, 1978, 1979), boosted cell yields considerably. In the presence of both the *Methanospirillum* and *Bacterioides*, the cell yield of *T. termopsidis* was increased roughly 15-fold. These preliminary data suggest that termite bacteria may be a better food source for cellulolytic protozoa than mixed rumen bacteria, and that the hydrogen-consuming activities of some bacteria (e.g. methanogens) might help to 'pull' anaerobic cellulose decomposition in the termite hindgut. The importance of methanogens as terminal organisms in anaerobic food webs has been pointed out by Wolfe (1979).

Methane is emitted from live termites, and methane bacteria have been isolated from hindguts of *R. flavipes*. The bacteria are rod-shaped

Table 7.1. *Effects of* Methanospirillum hungatii *and heat-killed termite gut bacteria on growth of* Trichomitopsis termopsidis in vitro[a]

Omitted from basal medium[b]	Added to basal medium[b]	Growth yield of *T. termopsidis* (cells ml^{-1})[c]	Presence of H_2 in headspace
—	—	3800 (23)	+
—	Msp	7800 (39)	−
AWRB	JW20	31 900 (31)	+
AWRB	Msp + JW20	58 000 (26)	−

Note:

[a] *T. termopsidis* strain 6057 was obtained from Dr M. A. Yamin.

[b] The basal medium and culture conditions were as described by Yamin (1978). Abbreviations: AWRB, autoclaved, washed rumen bacteria (Yamin, 1978); Msp, viable cells of *Methanospirillum hungatii* strain JF 1 (obtained from Dr M. P. Bryant); JW20, heat-killed cells of *Bacteroides* sp. strain JW20 isolated from hindguts of *Reticulitermes flavipes* termites (Schultz & Breznak, 1978).

[c] Determined by direct microscopic count. Numbers in parenthesis indicate the number of days required to reach maximum cell density. Initial cell densities were approximately 500 cells ml^{-1}.

and await complete characterization (Odelson and Breznak, unpublished). Rates of methane emission by termites vary, however, depending on the termite species and their diet prior to assay. Rates of emission as low as 0.3 nmole CH_4 hr^{-1} g fresh wt^{-1} were observed for *Coptotermes formosanus* worker larvae feeding on nest wood, whereas rates up to 73.1 nmole CH_4 hr^{-1} g fresh wt^{-1} were seen with *R. flavipes* under the same conditions (Breznak, 1975). When feeding on cellulose filter paper, rates of methane emission by *R. flavipes* increased to 1340 nmole hr^{-1} g fresh wt^{-1} (Breznak, 1975). Low amounts of methane emission were reported for *Marginitermes hubbardi* (LaFage & Nutting, 1979). Zimmerman, Greenberg, Wandiga & Crutzen (1982) found that methane was emitted from *Reticulitermes tibialis* (approximately 277 nmole hr^{-1} g fresh wt^{-1}) and *Gnathamitermes perplexus* (approximately 258 nmole hr^{-1} g fresh wt^{-1}) (values calculated from Zimmerman's data by assuming a fresh weight of 4 mg per termite). Based on these determinations, Zimmerman *et al.* (1982) suggested that of the total annual global production of methane ($3–12 \times 10^{14}$ g), termites could contribute as much as 1.5×10^{14} g. This estimate seems high, and it is based on analyses of only a few termite species as well as on termite population density data which are fairly limited (Wood & Sands, 1978). However, it is not unreasonable to assume that termites could make a significant contribution to local methane emissions, particularly at sites where they abound.

If polysaccharide dissimilation in the hindgut of lower termites is basically considered as the anaerobic fermentation of glycosyl units by the protozoa ($nC_6H_{12}O_6 + 2nH_2O \rightarrow 2nC_2H_4O_2 + 2nCO_2 + 4nH_2$), then a maximum of nCH_4 could be formed by methanogens from H_2 and CO_2 according to the reaction $nCO_2 + 4nH_2 \rightarrow nCH_4 + 2nH_2O$. Assuming all the acetate produced in the hindgut was then oxidized to CO_2 and H_2O, CH_4 emission by termites should be 20% that of total CO_2 emission on a molar basis. However, recent studies indicate that it is less than 1% for *R. flavipes* (Odelson & Breznak, 1983) and *R. tibialis* (Zimmerman *et al.*, 1982). Hydrogen not used in methanogenesis is not merely evolved, however. On a molar basis H_2 emission is only 10% that of CO_2 for *Zootermopsis nevadensis* (Gilmour, 1940), and it is usually 0.7–1.5% that of CO_2 for *R. flavipes* (Odelson & Breznak, 1983). Consequently, although some hydrogen escapes from the hindgut system, most of the reducing equivalents derived from cellulose fermentation are somehow consumed. Either free H_2 is not formed appreciably by protozoa *in situ*, or it is formed and rapidly used in reactions other than methanogenesis. One possible explanation is that hydrogen evolved from protozoa (and perhaps from other hindgut bacteria) is also consumed by *Acetobacterium*-type bacteria that are able to reduce CO_2 to acetate according to the reaction $2CO_2 + 4H_2 \rightarrow C_2H_4O_2 + 2H_2O$ (Balch, Schoberth, Tanner & Wolfe, 1977). Such bacteria could function along with methanogens as electron sink organisms and could also serve as an additional source of acetate for the termite. Moreover, use of H_2 and CO_2 for acetogenesis in the gut, with subsequent oxidation of acetate by the termite, would not alter the respiratory quotient of termites from the normal value of approximately 1.0 (Peakin & Josens, 1978). Acetogenesis from CO_2 appears to occur in other gastrointestinal ecosystems such as the caecum of guinea pigs, rats, and rabbits (Prins & Lankhorst, 1977), although it must be emphasized that such a reaction has not yet been confirmed for termite hindguts.

A number of bacterial isolates from lower termites have been found to possess C_x-type cellulase activity with carboxymethylcellulose as substrate (Krelinova, Kirku & Skoda, 1977; Thayer, 1976, 1978). However, at present there is no convincing evidence that bacteria are quantitatively important to the hydrolysis of crystalline cellulose *in vivo*; at least not in lower termites which possess cellulolytic protozoa (Breznak, 1975, 1982). Considering that many of the bacteria isolated from hindguts are fairly common heterotrophs (see above), one wonders what substrate (or substrates) supports their growth *in situ*. A clue may be found in the studies of Yamin (1980) and Hungate (1943) who observed that 10–30% of cellulose carbon fermented by hindgut protozoa *in vitro* could not be recovered as acetate and CO_2. Yamin suggested that some of the missing carbon might be present as soluble intermediates (oligomers) of cellulose hydrolysis released from the protozoa. It may be advantageous for protozoa to release hydrolysis intermediates *in situ*, because, if these could be used by the bacteria, the protozoa could essentially 'culture' a population of bacterial cells on which they might subsequently feed. The requirement for killed bacteria by axenic protozoan cultures (Yamin, 1978, 1981; Table 7.1), the early mixed culture studies conducted by Trager (1934), and the common occurrence of bacteria attached to (or within) hindgut protozoa (Ball, 1969), all support such speculation. Furthermore, Veivers, O'Brien & Slaytor (1982) found that metabolic activities of bacteria are important in maintaining anaerobic conditions in the gut of *Mastotermes darwiniensis*, and this may be an additional benefit to the protozoa, which are strict anaerobes.

Based on considerations discussed above, a working model is proposed for wood polysaccharide fermentation by the hindgut microbiota of lower termites (Fig. 7.3). The model is based on the original scheme

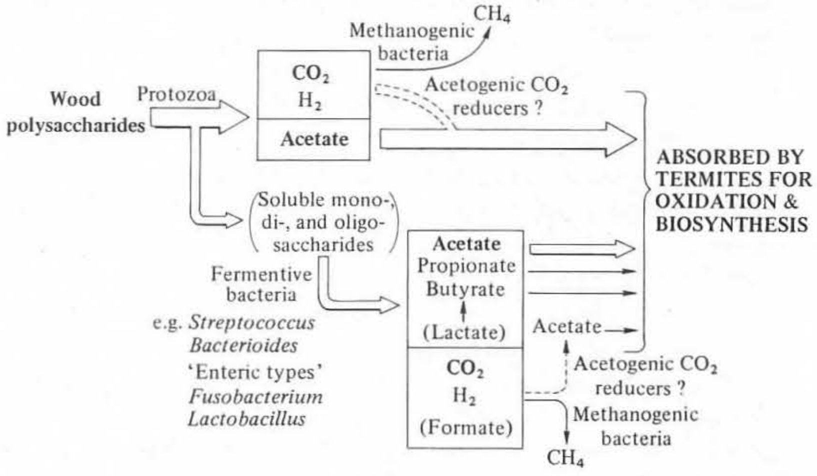

Fig. 7.3. Proposed working model for wood polysaccharide degradation in the hindgut of lower termites. Thickness of arrows represents approximate relative quantitative contribution of the respective reactions. Major products of the hindgut fermentation are indicated in boldface letters. See text for details.

postulated by Hungate (1939, 1943), but is modified to accommodate some of our current information on termite hindgut bacteria and metabolites present in the hindgut. According to this scheme, VFAs (principally acetate) are the major microbial metabolites supplied to the termites: these are readily oxidizable substrates for termites (Odelson & Breznak, 1983), and they are also known to be important precursors for synthesis of termite cuticular hydrocarbons (Blomquist, Howard & McDaniel, 1979), amino acids (Mauldin, Rich, & Cook, 1978), and terpenes (Prestwich, Jones & Collins, 1981). Most of the acetate is derived from fermentation by the protozoa. This is undoubtedly because the protozoa are very abundant in the hindgut and have the ability to endocytose, and thereby sequester, wood particles during the degradation. Consequently, most of the fermentation products formed should be those liberated by the protozoa and be dominated by acetate, CO_2 and H_2. Smaller amounts of products are thought to be derived from the bacteria, via the fermentation of mono-, di-, and soluble oligosaccharides released from protozoan cells. Fermentative bacteria that have been isolated from hindguts are known to ferment glucose and cellobiose (Schultz & Breznak, 1978), although none have been tested on soluble oligosaccharides of C_1 or greater. This is an important question which should be examined. Metabolites such as formate and lactate, which are known to be produced by pure cultures of termite hindgut bacteria (Schultz & Breznak, 1978), do not accumulate in the hindgut. In the case of lactate, *in vitro* co-culture studies by Schultz & Breznak (1979) have shown that lactate production by termite hindgut streptococci can support the growth of bacterioides, which ferment the compound to propionate, acetate, and CO_2. A similar type of cross-feeding of lactate is believed to occur *in situ*. If formate is produced in the gut, it is probably used as a methanogenic substrate.

The present scheme is derived mainly from studies of cellulose decomposition. However, virtually nothing is known about the mechanisms of the degradation of the other major polysaccharides of wood, i.e. the hemicelluloses. This is another important area which must be examined in greater detail.

Role of termite enzymes. The working model depicted in Fig. 7.3 is intended to describe the microbial decomposition of wood polysaccharides which reach the hindgut. It is not meant to imply that termite enzymes have no role in wood polysaccharide degradation. Indeed, many years ago Hungate (1938) found that *Zootermopsis* could digest approximately one third of the polysaccharides in wood without the aid of their hindgut protozoa. Lower termites have been shown to possess a variety of carbohydrases in their gut, including cellulases and β-glucosidases (LaFage & Nutting, 1978; McEwen, Slaytor & O'Brien, 1980; Mishra, 1980; O'Brien *et al.*, 1980; Veivers, Musca, O'Brien & Slaytor, 1982; Yamaoka & Nagatani, 1975; Yokoe, 1964), and there is some evidence that cellulase is synthesized by the termites themselves. However, most of such claims have been made by using carboxymethylcellulose or reprecipitated cellulose as the assay substrate. These substrates can be used to demonstrate the presence of C_x-type cellulases (active against noncrystalline cellulose and soluble derivatives or degradation products of cellulose), but not C_1-type cellulases (active against crystalline cellulose) (Wood & McCrae, 1979). On the other hand, Veivers, Musca, O'Brien & Slaytor (1982) showed the presence of C_1-cellulase in salivary glands and hindguts of *Mastotermes darwiniensis* where salivary enzyme activity appeared to be entirely of termite origin. Thus, it may well be that some cellulose hydrolysis can be initiated by enzymes of lower termites in the fore- and midgut. However, the importance of the hindgut microbiota (particularly the protozoa) to the survival of lower termites on sound wood or cellulose is undeniable.

Higher termites

Surprisingly little work has been done on the role of gut organisms in digestive processes of higher termites. Since the higher termites lack protozoa in their hindguts, it has been generally assumed that bacteria are primarily responsible for cellulose digestion in these insects. However, at present there is no good evidence to support that notion. Hungate (1946*b*) isolated an anaerobic, cellulolytic actinomycete (*Micromonospora propionici*) from the gut of *Amitermes minimus*, but he regarded the organism to be insignificant *in situ* because of its low population density in the gut and its slow growth rate *in vitro*. We have been unable to demonstrate cellulolytic bacteria in the gut of *Nasutitermes corniger*, using strict anaerobic techniques and ball-milled Whatman filter paper as a substrate (Breznak, unpublished). By contrast, higher termites appear to synthesize carbohydrases (McEwen *et al.*, 1980; O'Brien *et al.*, 1980; Kovoor, 1970; Potts & Hewitt, 1973), including cellulase active on crystalline cellulose (Potts & Hewitt, 1974). It may be that phylogenetically 'higher' termites are independent of their hindgut bacteria for cellulose hydrolysis. Nevertheless, a prominent fermentation appears to occur in the hindgut, and acetate and other VFAs have been identified in hindguts of *Microcerotermes edentatus* (Kovoor, 1967*b*) and *Nasutitermes corniger* (Odelson & Breznak, 1983). Perhaps fermentative activities of the gut bacteria, rather than cellulose hydrolysis *per se*, are important to the nutrition of higher termites. Clearly, more research on this group of termites is sorely needed.

The importance of mycophagy, as a source of nutrients and digestive enzymes for fungus-cultivating Macrotermitinae, is dealt with by Martin elsewhere in this volume (Chapter 6).

Role of gut microbes in termite nitrogen economy

The oligonitrotrophic habit of termites is most conspicuous for those species that thrive on sound, decay-free wood containing as little as 0.03–0.1% nitrogen on a dry weight basis (Cowling & Merrill, 1966) and exhibiting a C:N ratio of about 1000:1. Since termite tissues contain nitrogen in amounts similar to those of other animals (approximately 11% nitrogen on a dry weight basis; LaFage & Nutting, 1978; Potrikus & Breznak, 1980*a*), it would appear that termites have evolved efficient mechanisms for acquiring and/or conserving combined nitrogen. This seems to be true, and it appears that gut microbes can aid termites in both of these processes.

Nitrogen fixation

Nitrogen fixation by termites would constitute a means for acquisition of combined nitrogen, and it is a process that has been suspected ever since Cleveland's (1925*b*) claim that termites could live '... perhaps indefinitely on a diet of pure cellulose.' Only recently, however, has this suspicion received experimental support. By using the acetylene reduction assay, a reliable indicator of nitrogen fixation (Hardy, Burns & Holsten, 1973), nitrogen fixation has been inferred for a variety of termites (Table 7.2). Since it is a uniquely prokaryotic phenomenon, the fixation has generally been assumed to be mediated by bacteria, probably gut bacteria. This assumption appears to be valid, however, inasmuch as nitrogen fixation activity can be abolished by feeding termites antibacterial drugs (Breznak *et al.*, 1973). Moreover, nitrogen fixing bacteria have been isolated from termite guts. French, Turner & Bradbury (1976) isolated nitrogen fixing *Citrobacter freundii* strains from Australian termite species (*Coptotermes lacteus, Mastotermes darwiniensis*, and *Nasutitermes exitiosus*), whereas Potrikus &

Breznak (1977) isolated another member of the Enterobacteriaceae (*Enterobacter agglomerans*) from hindguts of *Coptotermes formosanus*.

In Table 7.2, the rates of nitrogen fixation predicted from acetylene reduction have been converted to a TDN equivalent. The latter represents 'time to double nitrogen' and is the amount of time it would take for a given termite species to double its nitrogen content, assuming that the measured fixation rates remained constant. A striking feature of these data is the wide variation of nitrogen fixation rates in termites, both interspecifically as well as intraspecifically. Considering these rates in *R. flavipes* workers for example, it would be difficult to argue that nitrogen fixation is important to this termite's nitrogen economy: particularly since the TDN equivalent is nearly 1000 years. A similar difficulty would be encountered with data from other species such as *Cubitermes* sp., *Labiotermes* sp., and *Zootermopsis* sp. By contrast, nitrogen fixation might be more important to termites such as *Cornitermes* sp., *I. minor*, or *T. trinervoides*, whose TDN values are far lower. In fact, for whole colonies of *N. corniger* the maximum rates of nitrogen fixation reported could allow for complete doubling of colony nitrogen in 0.5 years (Table 7.2). This certainly seems significant, although it would help greatly to know how fast termite populations increase *in the field*, and such data are scarce. Nevertheless, J.P.E.C. Darlington (cited by Prestwich & Bentley, 1981) estimates that *Macrotermes* species in East Africa turnover four populations annually. If *N. corniger* population growth is similar, then nitrogen fixation could conceivably contribute 50% of all the termite's nitrogen needs.

Several factors might account for the large variations in nitrogen fixation rates seen in Table 7.2. One of these is the nitrogen content of the food being eaten. For example, Breznak *et al.* (1973) found that acetylene reduction rates in *C. formosanus* varied inversely with the amount of combined nitrogen (e.g. KNO_3, $(NH_4)_2SO_4$, aspartic acid, or killed cells of *Escherichia coli*) added to a Whatman filter paper food disc. A significant change in fixation rates could be detected within five hours of a dietary shift, and variations in fixation rates up to 200-fold were observed. Inasmuch as nitrogenase synthesis by bacteria is repressed by readily utilizable sources of combined nitrogen (Brill, 1975), the modulation of nitrogen-fixing activity in *C. formosanus* presumably reflected a response of nitrogen-fixing gut bacteria to dietary nitrogen levels. Similarly, the difference in fixation rates between *R. perarmatus* and *N. corniger* was attributed to the fact that the former feeds on

Table 7.2. *Nitrogen fixation (acetylene reduction) rates of live termites*

Termites	*Caste*[a]	*g of N fixed g fresh* wt^{-1} day^{-1}[b]	*TDN* (yr)[c]	*Reference*
Amitermes sp.	W	0.36	126	Sylvester-Bradley, Bandeira & de Oliveira, 1978
	S	0.45	100	Sylvester-Bradley, Bandeira & de Oliveira, 1978
Armitermes sp.	W + S	1.44	31	Sylvester-Bradley, Bandeira & de Oliveira, 1978
Coptotermes formosanus	W	0.16–49.39	238–1	Breznak, 1975; Breznak, Brill, Mertins & Coppel, 1973
	S	0.03	1507	Breznak, Brill, Mertins & Coppel, 1973
Coptotermes lacteus	W	0.37–1.87	122–24	French, Turner & Bradbury, 1976
Cornitermes sp.	W + S	1.06	43	Sylvester-Bradley, Bandeira & de Oliveira, 1978
Cryptotermes brevis	BL	0.38	119	Breznak, Brill, Mertins & Coppel, 1973
Cubitermes sp.	W	0.17	265	Rohrmann & Rossman, 1980
	R	0.00	∞	Rohrmann & Rossman, 1980
Heterotermes sp.	W	0.94	48	Sylvester-Bradley, Bandeira & de Oliveira, 1978
	S	0.00	∞	Sylvester-Bradley, Bandeira & de Oliveira, 1978
Incisitermes minor	W	1.00–18.87	45–2	Benemann, 1973
	S	0.33	137	Benemann, 1973
	R	0.66	68	Benemann, 1973
Labiotermes sp.	W	0.16	282	Sylvester-Bradley, Bandeira & de Oliveira, 1978
Macrotermes ukuzii	W, S, R	0.00	∞	Rohrmann & Rossman, 1980
Mastotermes darwiniensis	W	0.00–23.47	∞–2	French, Turner & Bradbury, 1976
Nasutitermes corniger[d]	W	6.00–8.00	8–6	Prestwich & Bentley, 1981; Prestwich, Bentley & Carpenter, 1980
	S	0.90–28.40	50–2	Prestwich & Bentley, 1981; Prestwich, Bentley & Carpenter, 1980
	Whole colony	27.40–81.68	2–0.5	Prestwich & Bentley, 1981
Nasutitermes exitiosus	W	0.00–5.60	∞–8	French, Turner & Bradbury, 1976
Nautitermes sp.	W	0.20–13.28	226–3	Sylvester-Bradley, Bandeira & de Oliveira, 1978
	S	0.87–7.44	52–6	Sylvester-Bradley, Bandeira & de Oliveira, 1978
	W + S	1.11–18.31	41–2	Sylvester-Bradley, Bandeira & de Oliveira, 1978
Neocapritermes sp.	W	0.00	∞	Sylvester-Bradley, Bandeira & de Oliveira, 1978
Reticulitermes flavipes	W	0.05	904	Breznak, Brill, Mertins & Coppel, 1973
	S	0.02	2260	Breznak, Brill, Mertins & Coppel, 1973
Rhynchotermes perarmatus	W	3.5	13	Prestwich, Bentley & Carpenter, 1980
	S	0.5	90	Prestwich, Bentley & Carpenter, 1980
Trinervitermes trinervoides	W	6.86	7	Rohrmann & Rossman, 1980
	S	4.48–4.73	10–9	Rohrmann & Rossman, 1980
	R	0.00	∞	Rohrmann & Rossman, 1980
Zootermopsis sp.	W+ BL	0.06	753	Breznak, Brill, Mertins & Coppel, 1973

Note:

[a] W, Workers; S, soldiers; BL, brachypterous larvae; R, reproductives.

[b] Values calculated assuming that N_2 fixation rates are one third that of acetylene reduction (Hardy, Burns & Holsten, 1973), and that fresh weight of termites = 6.7 × dry weight.

[c] TDN, time required for termites to double their nitrogen content (see text). It is assumed that the nitrogen content of all termites is 11% (dry wt basis; Potrikus & Breznak, 1980*a*).

[d] Mistakenly identified as *Nasutitermes ephratae* (Prestwich, Bentley & Carpenter, 1980) and corrected in a subsequent paper (Prestwich & Bentley, 1981).

material higher in combined nitrogen (leaf litter) than does the latter (which feeds on wood litter) (Prestwich, Bentley & Carpenter, 1980).

The feeding preferences of some termites may in fact obviate the need for nitrogen fixation, simply because their diets contain adequate, albeit low, amounts of combined nitrogen. For example, many of the Hodo-, Rhino-, and Kalotermitidae actually prefer wood that has undergone some decay by fungi (Lee & Wood, 1971). Experiments by Hungate (1941, 1944) suggested that fungi have the effect of increasing the relative amount of combined nitrogen in wood, by degrading the polysaccharide components and by translocating nitrogen into the resource from the surroundings (e.g. soil). Thus, Hungate's (1941, 1944) nitrogen balance studies on *Zootermopsis, Incisitermes* (*Kalotermes*), and *Reticulitermes* species, which fed preferentially on decayed portions of wood, showed no evidence for nitrogen fixation. Perhaps it is not surprising that these species also show only low to moderate rates of nitrogen fixation when assayed by the acetylene reduction method (Table 7.2). It is interesting that termites are not the only animals whose nitrogen fixation rates appear to vary inversely with dietary nitrogen: a similar response has been observed with sea urchins (Guerinot, Fong & Patriquin, 1977) and even man (Bergersen & Hipsley, 1970).

Age or developmental stage of termites may also have a bearing on rates of nitrogen fixation and may account for some of the intraspecific variations seen in Table 7.2. For example, Breznak (1975) found that small worker larvae of *C. formosanus* had rates 300-fold greater than larger, more fully developed workers. Presumably, the nitrogen needs of the smaller workers were greater, and this was reflected in their elevated nitrogen fixation rates. An inverse relationship between nitrogen fixation rate and body size has also been observed with marine shipworms (Carpenter & Culliney, 1975). Other factors that could contribute to intraspecific variations in fixation rates are cyclic demands for nitrogen placed on colony members during bursts of reproductive activity (Prestwich & Bentley, 1981), as well as physical disturbances to the termites during their manipulation for assay (Potrikus & Breznak, 1977; Prestwich & Bentley, 1981; Prestwich *et al.*, 1980).

Nitrogen conservation

The complementary side to nitrogen acquisition is nitrogen conservation and there are three main ways this could be done: storage/recycling of nitrogenous metabolic wastes; recycling of termite tissues (e.g. exoskeletons); and digestion and assimilation of gut microbes or lytic or secretory products thereof. The contribution of these processes to termite nitrogen economy has been largely speculative. However, recent evidence suggests that recycling of nitrogenous wastes by termite gut bacteria is an important conservation mechanism.

Uric acid (UA) is a common, and well-suited, nitrogenous excretory product of a variety of terrestrial insects (Cochran, 1975). Because of its poor solubility in aqueous solution, UA can be voided with faeces as a non-toxic solid, thereby minimizing water loss to the insect. However, Leach & Granovsky (1938) speculated that in termites UA might be degraded by hindgut organisms to a form of nitrogen reusable by the insects. For more than 40 years this hypothesis remained untested, until Potrikus & Breznak (1980*a*, *b*, *c*; 1981) critically examined its validity. Working mainly with *Reticulitermes flavipes*, we found that termite tissues contain UA (Potrikus & Breznak, 1980*a*), as well as key enzymes for UA biosynthesis (purine nucleoside phosphorylase, EC 2.4.2.1, and xanthine dehydrogenase, EC 1.2.1.37; Potrikus & Breznak, 1981). However, little or no UA was voided in faeces (Potrikus & Breznak, 1980*a*), despite the fact that termite tissues did not possess a uricase or any other enzyme to degrade UA (Potrikus & Breznak, 1980*a*; 1981). This apparent paradox was reconciled by the demonstration that uricolysis did, in fact, occur in *R. flavipes*, but as an anaerobic process mediated by hindgut bacteria (Potrikus & Breznak, 1980*b*, *c*; 1981). Major uricolytic isolates were *Streptococcus* sp., *Bacteroides termitidis* and *Citrobacter* sp. All isolates used UA as an energy source for growth, and major products of UA fermentation were NH_3, CO_2, and acetate. Uric acid decomposition by the bacteria was a strictly anaerobic processes, not only for *B. termitidis* which is a strict anaerobe, but for uricolytic streptococci and citrobacters as well. As expected, uricolytic activity of gut homogenates was also inhibited by exposure to air and was markedly reduced in termites that had been fed antibacterial drugs.

By using ^{14}C- and ^{15}N-labelled UA, Potrikus & Breznak (1981) were able to show that UA was transported from its site of synthesis and storage (fat body tissue) to the gut by Malpighian tubules, and that microbial uricolysis *in situ* liberates nitrogen that is reused by the termites for biosynthesis.

Based on these results a model for microbial-mediated UA-nitrogen (UA-N) recycling in *R. flavipes* can be proposed (Fig 7.4). The precise form of UA-N taken up by termites is unknown, however. Ammonia is one possibility since it is the major nitrogenous product of uricolysis by the gut bacteria in pure culture (Potrikus & Breznak, 1980*a*), and termite tissues possess glutamine synthetase (EC 6.3.1.2; Potrikus & Breznak, 1981). Alternatively, UA-N might first be assimilated by gut microbes to become available to termites as organic nitrogen, either by secretion from gut microbes, or by lysis of cells in the gut, or through coprophagy or trophallaxis. However, at present virtually nothing is known about the dynamics of UA synthesis and mobilization in *R. flavipes* under natural conditions, although it seems safe to assume that UA biosynthesis will be favoured when the intake of dietary nitrogen by termites exceeds that needed for biosynthesis, whereas UA mobilization will be favoured when colony demand for nitrogen is high (e.g. during peaks of reproductive activity). Nevertheless, UA-N recycling is almost certainly significant to the nitrogen economy of *R. flavipes*. It was

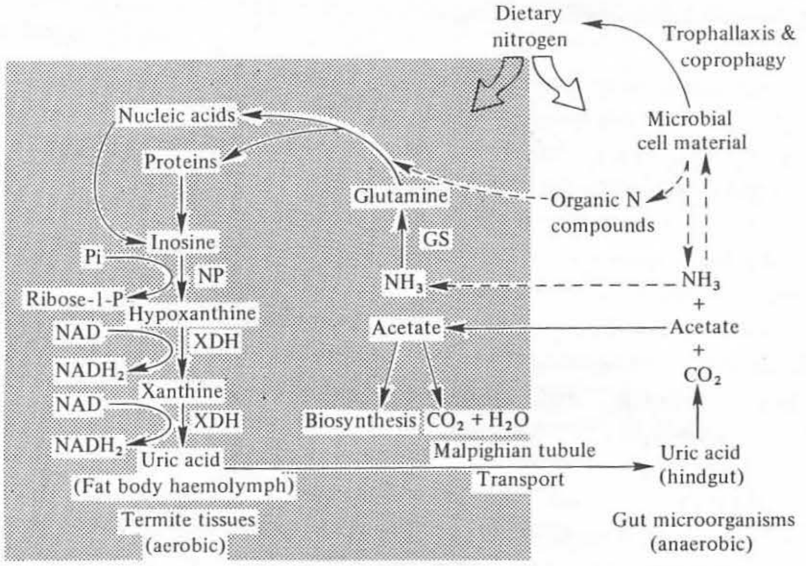

Fig. 7.4. Proposed working model for recycling of uric acid nitrogen in *Reticulitermes flavipes*. Stippled portion depicts reactions mediated by termite enzymes and tissues, whereas the clear portion depicts reactions mediated by hindgut bacteria under anaerobic conditions. Solid arrows indicate reactions for which there is experimental proof or which are extremely likely, whereas dashed arrows depict reactions which are still speculative. Abbreviations: GS, glutamine synthetase; NP, purine nucleoside phosphorylase; Pi, phosphate ion; XDH, xanthine dehydrogenase. See text for details. (Modified from Potrikus, 1980.)

calculated that biosynthesis of UA was an energetically sound investment for *R. flavipes*, and that bacterial uricolysis could liberate enough nitrogen annually to support biosynthesis of termites equivalent to 30% of the colony biomass (Potrikus & Breznak, 1981). Thus, in termites UA may not function as a waste product *per se*, but could be an important storage form of nitrogen. Such a situation appears to hold for cockroaches: in an elegant study with *Periplaneta americana*, Mullins & Cochran (1975*a*, *b*) showed that UA accumulated by the insects on high nitrogen diets was mobilized when shifted to a low nitrogen diet. Part of the UA-N mobilized under such conditions was used for oothecal production by females. However, microbial involvement in cockroach uricolysis is unclear (see Cochran, 1975 for review of this issue). By contrast, Nazarczuk, O'Brien & Slaytor (1981) found that *Nasutitermes exitiosus* and *Coptotermes lacteus* failed to mobilize UA over a seven week period when starved, or when their gut bacteria were killed with tetracycline. However, if fully developed workers were used in these experiments, their nitrogen needs may have been very low and limited to that needed for protein turnover. Consequently, it may not be surprising that UA was not mobilized under these conditions. It would be interesting to start examining nitrogen metabolism in termites under laboratory conditions that mimic nutritional stresses expected in nature, e.g. by using test groups of termites containing young, nutrient-dependent larvae.

Another potentially important strategy for nitrogen conservation in termite colonies would be the consumption of exuviae, as well as dead, dying, or supernumerary individuals (via cannibalism). The incidence of such behaviour has been reviewed by LaFage & Nutting (1978). A rich source of nitrogen in such food is chitin, and chitinase has in fact been demonstrated in termites (Tracy & Youatt, 1958; Waterhouse, Hackman & McKellar, 1961). However, since whole insects were used to prepare the enzyme extracts, the anatomical origin of such activity is unknown. By contrast, Rohrmann & Rossman (1980) found that most of the chitinase of *Macrotermes ukuzii* workers and nymphs was gut-associated. Since *M. ukuzii* cultivates and consumes a symbiotic *Termitomyces* fungus, a digestive chitinase may make fungal cell wall chitin available to this termite as a nitrogen source. These investigators also isolated a chitinolytic bacterium from the gut of *M. ukuzii*, but they were uncertain of the contribution of the bacterium to chitinase activity of gut homogenates. It would be interesting to examine termite gut microbes further as a source of chitinase.

It is not yet known if termites can conserve nitrogen by actively digesting some of their gut microbiota. While lysis of protozoa in the hindgut of lower termites appears insignificant as a means of nitrogen conservation (Hungate, 1955), proctodeal trophallaxis could conserve microbial nitrogen if cells could be digested in the gut of recipients. Rates of growth, lysis, and turnover of bacteria in the hindgut are unknown, but would add greatly to our understanding of the nutritive potential of bacterial cell material. On the other hand, the ability of termite hindgut bacteria to supply amino acids to their host is suggested by two studies. Mauldin *et al.* (1978) found that normal synthesis of amino acids by *Coptotermes formosanus* fed ^{14}C-acetate was dependent on the combined presence of normal gut bacteria and two protozoa (*Holomastigotoides hartmanni* and *Spirotrichonympha leidyi*). A similar role for bacteria was suggested by Speck, Becker & Lenz (1971), who used *Reticulitermes santonensis* fed ^{14}C-glucose.

Lignin degradation in termites

The question of lignin degradation by termites is intriguing, since much of the termite gut is anaerobic and natural anaerobic mechanisms for lignin degradation are unknown. Conclusions based on analysis of termite faeces are conflicting, with some authors reporting as much as 83% lignin degradation (reviewed by LaFage & Nutting, 1978; Lee & Wood, 1971), and others reporting virtually none (Esenther & Kirk, 1974). The controversy concerning lignin degradation has recently been discussed (Breznak, 1982; O'Brien & Slaytor, 1982) and need not be repeated in detail here. However, the work of Butler & Buckerfield (1979) must be cited as one of the most convincing arguments for lignin digestion in the termite gut. These investigators found that the higher termite *Nasutitermes exitiosus* readily respired ^{14}C-labelled lignins. Fourteen to 32% and 15–63% of the ^{14}C-label was evolved from synthetic and maize lignins respectively, which were labelled in various positions in the polymer (methoxy; C_2; ring). An important control was their demonstration that maximum $^{14}CO_2$ emission required the presence of live termites in the incubation vessels; little or no $^{14}CO_2$ was evolved when termites were removed. This indicated that lignin degradation was occurring *within* the termites' bodies, and not from voided faecal pellets. The specific site of degradation in the gut was not determined, nor was the involvement of gut microbes in the process.

However, Butler & Buckerfield (1979) speculated that the polymer might be degraded in the gut to smaller molecular weight derivatives which might be taken up through the gut epithelium and oxidized aerobically by termite tissues.

Concluding remarks

A significant amount of information has been obtained in the last decade or so on the biochemical activities of the termite gut microbiota and the symbiotic interaction between the microbiota and its host. We are now in a position to refine pre-existing models for such symbioses (e.g. Figs. 7.3, 7.4) and to begin to test the generality of these models. Particularly satisfying is the availability in pure culture of some of the dominant and seemingly important gut microbes of termites, including the anaerobic cellulolytic protozoa of lower termites. The biochemistry of such organisms will be an important topic for future research. Nevertheless, some quantitatively significant gut symbionts of termites remain to be isolated and studied, notably the spirochaetes. It seems certain that a better understanding of the physiology, biochemistry, and nutrition of such forms will greatly increase our understanding of the gut ecosystem from which they came and their importance to termite nutrition.

We now also have a clearer notion of the termites' own biochemical capabilities, and it is beginning to look as though higher termites might be independent of their gut microbes, at least with regard to the initial steps of cellulose degradation. Further studies must continue to discriminate carefully the termites' capabilities from those provided by the gut microbiota. This is of utmost importance as we probe more deeply into the biochemistry of polysaccharide and lignin degradation, and nitrogen metabolism, in these ecologically important insects.

Acknowledgments. I am grateful to R. W. O'Brien and P. R. Zimmerman for providing me with preprints of unpublished papers. Some of the research reported in this review was conducted by the writer with support from the National Science Foundation (BMS 75-05850, PCM 77-05732, and PCM 80-12026) and the Michigan Agricultural Experiment Station of Michigan State University, and for which he is grateful. This is journal article no. 10609 from the Michigan Agricultural Experiment Station.

References

Balch, W. E., Schoberth, S., Tanner, R. S. & Wolfe, R. S. (1977). *Acetobacterium*, a new genus of hydrogen-oxidizing, carbon dioxide-reducing, anaerobic bacteria. *International Journal of Systematic Bacteriology*, **27**, 355–61.

Ball, G. H. (1969). Organisms living on and in protozoa. In *Research in Protozoology*, vol. 3, ed. T.-T. Chen, pp. 565–718. New York: Pergamon Press.

de Bary, A. (1879). *Die Erscheinung der Symbiose*. Strasburg: Trubner.

Benemann, J. R. (1973). Nitrogen fixation in termites. *Science*, **181**, 164–5.

Bergerson, F. J. & Hipsley, E. H. (1970). The presence of N_2-fixing bacteria in the intestines of man and animals. *Journal of General Microbiology*, **60**, 61–5.

Bignell, D. E. & Anderson, J. M. (1980). Determination of pH and oxygen status in the guts of lower and higher termites. *Journal of Insect Physiology*, **26**, 183–8.

Bignell, D. E., Oskarsson, H. & Anderson, J. M. (1979). Association of actinomycete-like bacteria with soil-feeding termites (Termitidae, Termitinae). *Applied and Environmental Microbiology*, **37**, 339–42.

Bignell, D. E., Oskarsson, H. & Anderson, J. M. (1980*a*). Distribution and abundance of bacteria in the gut of a soil-feeding termite *Procubitermes aburiensis* (Termitidae, Termitinae). *Journal of General Microbiology*, **117**, 393–403.

Bignell, D. E., Oskarsson, H. & Anderson, J. M. (1980*b*). Specialization of the hindgut wall for the attachment of symbiotic micro-organisms in a termite *Procubitermes aburiensis* (Isoptera, Termitidae, Termitinae). *Zoomorphology*, **96**, 103–12.

Bignell, D. E., Oskarsson, H. & Anderson, J. M. (1980*c*). Colonization of the epithelial face of the peritrophic membrane and the ectoperitrophic space by actinomycetes in a soil-feeding termite. *Journal of Invertebrate Pathology*, **36**, 426–8.

Blomquist, G. J., Howard, R. W. & McDaniel, C. A. (1979). Biosynthesis of the cuticular hydrocarbons of the termite *Zootermopsis angusticollis* (Hagen). Incorporation of propionate into dimethylalkanes. *Insect Biochemistry*, **9**, 371–4.

Bloodgood, R. A. (1975). Ultrastructure of the attachment of *Pyrsonympha* to the hindgut wall of *Reticulitermes tibialis*. *Journal of Insect Physiology*, **21**, 391–9.

Breznak, J. A. (1973). Biology of nonpathogenic, host-associated spirochetes. *CRC Critical Reviews in Microbiology*, **2**, 457–89.

Breznak, J. A. (1975). Symbiotic relationships between termites and their intestinal microbiota. In *Symbiosis: 29th Symposium of the Society for Experimental Biology*, ed. D. H. Jennings & D. L. Lee, pp. 559–80. Cambridge University Press.

Breznak, J. A. (1982). Intestinal microbiota of termites and other xylophagous insects. *Annual Review of Microbiology*, **36**, 323–43.

Breznak, J. A. (1983). Hindgut spirochetes of termites and *Cryptocercus punctulatus*. In *Bergey's Manual of Systematic Bacteriology*, ed. N. R. Krieg (in press). Baltimore: Williams & Wilkins.

Breznak, J. A., Brill, W. J., Mertins, J. W. & Coppel, H. C. (1973). Nitrogen fixation in termites. *Nature, London*, **244**, 577–80.

Breznak, J. A. & Pankratz, H. S. (1977). In situ morphology of the gut microbiota of wood-eating termites [*Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki]. *Applied and Environmental Microbiology*, **33**, 406–26.

Brian, M. V. (ed.) (1978). *Production Ecology of Ants and Termites*. Cambridge University Press.

Brill, W. J. (1975). Regulation and genetics of bacterial nitrogen fixation. *Annual Review of Microbiology*, **29**, 109–29.

Butler, J. H. A. & Buckerfield, J. C. (1979). Digestion of lignin by termites. *Soil Biology and Biochemistry*, **11**, 507–11.

Carpenter, E. J. & Culliney, J. L. (1975). Nitrogen fixation in marine shipworms. *Science*, **187**, 551–2.

Cleveland, L. R. (1923). Symbiosis between termites and their intestinal protozoa. *Proceedings of the National Academy of Sciences of the USA*, **9**, 424–8.

Cleveland, L. R. (1924). The physiological and symbiotic relationships between the intestinal protozoa of termites and their host, with special reference to *Reticulitermes flavipes* Kollar. *Biological Bulletin*, **46**, 178–227.

Cleveland, L. R. (1925*a*). The effects of oxygenation and starvation on the symbiosis between the termite, *Termopsis*, and its intestinal flagellates. *Biological Bulletin*, **48**, 309–26.

Cleveland, L. R. (1925*b*). The ability of termites to live perhaps indefinitely on a diet of pure cellulose. *Biological Bulletin*, **48**, 289–93.

Cleveland, L. R. & Grimstone, A. V. (1964). The fine structure of the flagellate *Mixotricha paradoxa* and its associated micro-organisms. *Proceedings of the Royal Society of London, Series B*, **159**, 668–86.

Cochran, D. G. (1975). Excretion in insects. In *Insect Biochemistry and Function*, ed. D. J. Candy & B. A. Kilby, pp. 178–281. London: Chapman & Hall.

Cook, S. F. (1943). Nonsymbiotic utilization of carbohydrates by the termite *Zootermopsis angusticollis*. *Physiological Zoology*, **16**, 123–8.

Cowling, E. B. & Merrill, W. (1966). Nitrogen in wood and its role in wood deterioration. *Canadian Journal of Botany*, **44**, 1539–54.

Esenther, G. R. & Kirk, T. K. (1974). Catabolism of aspen sapwood in *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *Annals of the Entomological Society of America*, **67**, 989–91.

Eutick, M. L., Veivers, P., O'Brien, R. W. & Slaytor, M. (1978). Dependence of the higher termite, *Nasutitermes exitiosus* and the lower termite, *Coptotermes lacteus* on their gut flora. *Journal of Insect Physiology*, **24**, 363–8.

French, J. R. J., Turner, G. L. & Bradbury, J. F. (1976). Nitrogen fixation by bacteria from the hindgut of termites. *Journal of General Microbiology*, **95**, 202–6.

Gilmour, D. (1940). The anaerobic gaseous metabolism of the termite, *Zootermopsis nevadensis* Hagen. *Journal of Cellular and Comparative Physiology*, **15**, 331–42.

Guerinot, M. L., Fong, W. & Patriquin, D. G. (1977). Nitrogen fixation (acetylene reduction) associated with sea urchins (*Strongylocentrotus droebachiensis*) feeding on seaweeds and eelgrass. *Journal of the Fisheries Research Board of Canada*, **34**, 416–20.

Hardy, R. W. F., Burns, R. C. & Holsten, R. D. (1973). Applications of the acetylene–ethylene assay for measurement of nitrogen fixation. *Soil Biology and Biochemistry*, **5**, 47–81.

Honigberg, B. M. (1970). Protozoa associated with termites and their role in digestion. In *Biology of Termites*, vol. 2, ed. K. Krishna & F. M. Weesner, pp. 1–36. New York: Academic Press.

Hungate, R. E. (1938). Studies on the nutrition of *Zootermopsis*. II. The relative importance of the termite and the protozoa in wood digestion. *Ecology*, **19**, 1–25.

Hungate, R. E. (1939). Experiments on the nutrition of *Zootermopsis*. III. The anaerobic carbohydrate dissimilation by the intestinal protozoa. *Ecology*, **20**, 230–45.

Hungate, R. E. (1941). Experiments on the nitrogen economy of termites. *Annals of the Entomological Society of America*, **34**, 467–89.

Hungate, R. E. (1943). Quantitative analysis on the cellulose fermentation by termite protozoa. *Annals of the Entomological Society of America*, **36**, 730–9.

Hungate, R. E. (1944). Termite growth and nitrogen utilization in laboratory cultures. *Texas Academy of Sciences Proceedings and Transactions*, **27**, 1–7.

Hungate, R. E. (1946*a*). The symbiotic utilization of cellulose. *Journal of Elisha Mitchell Scientific Society*, **62**, 9–24.

Hungate, R. E. (1946*b*). Studies on cellulose fermentation. II. An anaerobic cellulose-decomposing actinomycete, *Micromonospora propionici*, n. sp. *Journal of Bacteriology*, **51**, 51–6.

Hungate, R. E. (1955). Mutualistic intestinal protozoa. In *Biochemistry and Physiology of Protozoa*, vol. 2, ed. S. H. Hutner & A. Lwoff, pp. 159–99. New York: Academic Press.

Hungate, R. E. (1975). The rumen microbial ecosystem. *Annual Review of Ecology and Systematics*, **6**, 39–66.

Kovoor, J. (1959). Anatomie du tractus intestinal dans le genre *Microcerotermes* (Silvestri), (Isoptera, Termitidae). *Bulletin de la Société zooligique de France*, **84**, 445–57.

Kovoor, J. (1967*a*). Etude radiographique du transit intestinal chez un termite supérieur. *Experientia*, **23**, 820–1.

Kovoor, J. (1967*b*). Présence d'acides gras volatils dans la panse d'un termite supérieur (*Microcerotermes edentatus* Was., Amitermitinae). *Comptes rendus hebdomadaire des séances de L'Académie des sciences*, **264D**, 486–8.

Kovoor, J. (1968). L'intestin d'un termite supérieur (*Microcerotermes edentatus*, Wasman, Amitermitinae). Histophysiologie et flore bactérienne symbiotique. *Bulletin biologique de la France et de la Belgique*, **102**, 45–84.

Kovoor, J. (1970). Présence d'enzymes cellulolytiques dans l'intestin d'un termite supérieur, *Microcerotermes edentatus* (Was.). *Annales des sciences naturelles (Zoologie)*, **12**, 65–71.

Krasil'nikov, N. A. & Satdykov, S. I. (1969). Estimation of the total bacteria in the intestines of termites. *Microbiology*, **38**, 289–92.

Krelinova, D., Kirku, V. & Skoda, J. (1977). The cellulolytic activity of some intestinal bacteria of termites. *International Biodeterioration Bulletin*, **13**, 81–7.

Krishna, K. (1969). Introduction. In *Biology of Termites*, vol. 1, ed. K. Krishna & F. M. Weesner, pp. 1–17. New York: Academic Press.

Krishna, K. & Weesner, F. M. (1969–1970). *Biology of Termites*, vols. 1 and 2. New York: Academic Press.

LaFage, J. P. & Nutting, W. L. (1978). Nutrient dynamics of termites. In *Production Ecology of Ants and Termites*, ed. M. V. Brian, pp. 165–232. Cambridge University Press.

LaFage, J. P. & Nutting, W. L. (1979). Respiratory gas exchange in the dry wood termite, *Marginitermes hubbardi* (Banks) (Isoptera: Kalotermitidae). *Sociobiology*, **4**, 257–67.

Leach, J. G. & Granovsky, A. A. (1938). Nitrogen in the nutrition of termites. *Science*, **87**, 66–7.

Lee, K. E. & Wood, J. G. (1971). *Termites and Soils*. New York: Academic Press.

McEwen, S. E., Slaytor, M. & O'Brien, R. W. (1980). Cellobiase activity in three species of Australian termites. *Insect Biochemistry*, **10**, 563–7.

McMahan, E. A. (1969). Feeding relationships and radioisotope techniques. In *Biology of Termites*, vol. 1, ed. K. Krishna & F. M. Weesner, pp. 387–406. New York: Academic Press.

Martin, M. M. & Martin, J. S. (1979). The distribution and origins of the cellulolytic enzymes of the higher termite, *Macrotermes natalensis*. *Physiological Zoology*, **52**, 11–21.

Mauldin, J. K. & Rich, N. M. (1980). Effect of chlortetracycline and other antibiotics on protozoan numbers in the eastern subterranean termite. *Journal of Economic Entomology*, **73,** 123–8.

Mauldin, J. K., Rich, N. M. & Cook, D. W. (1978). Amino acid synthesis from ^{14}C-acetate by normally and abnormally faunated termites, *Coptotermes formosanus*. *Insect Biochemistry*, **8,** 105–9.

Mishra, S. C. (1980). Carbohydrases in *Neotermes basei* Snyder (Isoptera: Kalotermitidae). *Material und Organismen*, **15,** 253–61.

Mullins, D. E. & Cochran, D. G. (1975*a*). Nitrogen metabolism in the American cockroach. I. An examination of positive nitrogen balance with respect to uric acid stores. *Comparative Biochemistry and Physiology*, **50A,** 489–500.

Mullins, D. E. & Cochran, D. G. (1975*b*). Nitrogen metabolism in the American cockroach. II. An examination of negative nitrogen balance with respect to mobilization of uric acid stores. *Comparative Biochemistry and Physiology*, **50A,** 501–10.

Nazarczuk, R. A., O'Brien, R. W. & Slaytor, M. (1981). Alterations of the gut microbiota and its effect on nitrogen metabolism in termites. *Insect Biochemistry*, **11,** 267–75.

Noirot, C. & Noirot-Timothée, C. (1967). L'epithélium absorbant de la panse d'un termite supérieur. Ultrastructures et rapport avec la symbiose bactérienne. *Annales de la Société entomologique de France*, **3,** 577–92.

Noirot, C. & Noirot-Timothée, C. (1969). The digestive system. In *Biology of Termites*, vol. 1, ed. K. Krishna & F. M. Weesner, pp. 49–88. New York: Academic Press.

O'Brien, G. W., Veivers, P. C., McEwen, S. E., Slaytor, M. & O'Brien, R. W. (1979). The origin and distribution of cellulase in the termites, *Nasutitermes exitiosus* and *Coptotermes lacteus*. *Insect Biochemistry*, **9,** 619–25.

O'Brien, R. W. & Slaytor, M. (1982). Role of microorganisms in the metabolism of termites. *Australian Journal of Biological Sciences*, **35,** 239–62.

Odelson, D. A. & Breznak, J. A. (1983). Volatile fatty acid production by the hindgut microbiota of xylophagous termites. *Applied and Environmental Microbiology*, **45,** 1602–13.

Peakin, G. J. & Josens, G. (1978). Respiration and energy flow. In *Production Ecology of Ants and Termites*, ed. M. V. Brian, pp. 111–63. Cambridge University Press.

Potrikus, C. J. (1980). 'Uric acid and uricolytic gut bacteria in wood-eating termites: a strategy for nitrogen conservation.' Unpublished Ph.D. dissertation, Michigan State University.

Potrikus, C. J. (1983). Role of microorganisms in the nitrogen economy of termites. *Bioscience* (in press).

Potrikus, C. J. & Breznak, J. A. (1977). Nitrogen-fixing *Enterobacter agglomerans* isolated from guts of wood-eating termites. *Applied and Environmental Microbiology*, **33,** 392–9.

Potrikus, C. J. & Breznak, J. A. (1980*a*). Uric acid in wood-eating termites. *Insect Biochemistry*, **10,** 19–27.

Potrikus, C. J. & Breznak, J. A. (1980*b*). Uric acid-degrading bacteria in guts of termites [*Reticulitermes flavipes* (Kollar)]. *Applied and Environmental Microbiology*, **40,** 117–24.

Potrikus, C. J. & Breznak, J. A. (1980*c*). Anaerobic degradation of uric acid by gut bacteria of termites. *Applied and Environmental Microbiology*, **40,** 125–32.

Potrikus, C. J. & Breznak, J. A. (1981). Gut bacteria recycle uric acid nitrogen in termites: a strategy for nutrient conservation. *Proceedings of the National Academy of Sciences of the USA*, **78,** 4601–5.

Potts, R. C. & Hewitt, P. H. (1973). The distribution of intestinal bacteria and cellulase activity in the harvester termite *Trinervitermes trinervoides* (Nasutitermitinae). *Insectes sociaux*, **20,** 215–20.

Potts, R. C. & Hewitt, P. H. (1974). Some properties and reaction characteristics of the partially purified cellulase from the termite *Trinervitermes trinervoides* (Nasutitermitinae). *Comparative Biochemistry and Physiology*, **47B,** 327–37.

Prestwich, G. D. & Bentley, B. L. (1981). Nitrogen fixation by intact colonies of the termite *Nasutitermes corniger*. *Oecologia*, **49,** 249–51.

Prestwich, G. D., Bentley, B. L. & Carpenter, E. J. (1980). Nitrogen sources for neotropical nasute termites: fixation and selective foraging. *Oecologia*, **46,** 397–401.

Prestwich, G. D., Jones, R. W. & Collins, M. S. (1981). Terpene biosynthesis by nasute termite soldiers (Isoptera: Nasutitermitinae). *Insect Biochemistry*, **11,** 331–6.

Prins, R. A. & Lankhorst, A. (1977). Synthesis of acetate from CO_2 in the cecum of some rodents. *FEMS Microbiology Letters*, **1,** 255–8.

Rohrmann, G. F. & Rossman, A. Y. (1980). Nutrient strategies of *Macrotermes ukuzii* (Isoptera: Termitidae). *Pedobiologia*, **20,** 61–73.

Sands, W. A. (1969). The association of termites and fungi. In *Biology of Termites*, vol. 1, ed. K. Krishna & F. M. Weesner, pp. 495–524. New York: Academic Press.

Schultz, J. E. & Breznak, J. A. (1978). Heterotrophic bacteria present in hindguts of wood-eating termites [*Reticulitermes flavipes* (Kollar)]. *Applied and Environmental Microbiology*, **35,** 930–6.

Schultz, J. E. & Breznak, J. A. (1979). Cross-feeding of lactate between *Streptococcus lactis* and *Bacteroides* sp. isolated from termite hindguts. *Applied and Environmental Microbiology*, **37,** 1206–10.

Speck, U., Becker, G. & Lenz, M. (1971). Ernährungsphysiologische Untersuchungen an termiten nach selektiver medikamentöser Ausschaltung der Darmsymbionten. *Zeitschrift für angewandte Zoologie*, **58,** 475–91.

Sylvester-Bradley, R., Bandeira, A. G. & de Oliveira, L. A. (1978). Fixacão de nitrogênio (reducão de acetileno) em cupins (Insecta: Isoptera) da Amazônia Central. *Acta Amazonica*, **8,** 621–7.

Thayer, D. W. (1976). Facultative wood-digesting bacteria from the hindgut of the termite *Reticulitermes hesperus*. *Journal of General Microbiology*, **95,** 287–96.

Thayer, D. W. (1978). Carboxymethylcellulase produced by facultative bacteria from the hind-gut of the termite *Reticulitermes hesperus*. *Journal of General Microbiology*, **106,** 13–18.

To, L. P., Margulis, L., Chase, D. & Nutting, W. L. (1980). The symbiotic microbial community of the Sonoran Desert termite: *Pterotermes occidentis*. *Biosystems*, **13,** 109–37.

To, L. P., Margulis, L. & Cheung, A. T. W. (1978). Pillotinas and hollandinas: distribution and behaviour of large spirochetes symbiotic in termites. *Microbios*, **22,** 103–33.

Tracy, M. V. & Youatt, G. (1958). Cellulase and chitinase in two species of Australian termites. *Enzymologia*, **19,** 70–2.

Trager, W. (1932). A cellulase from the symbiotic intestinal flagellates of termites and of the roach, *Cryptocercus punctulatus*. *Biochemical Journal*, **26,** 1762–71.

Trager, W. (1934). The cultivation of a cellulose-digesting flagellate, *Trichomonas termopsidis* a ozoa. *Biological Bulletin*, **66,** 182–90

Veivers, P. C., Musca, A. N., O'Brien, R. W. & Slaytor, M. (1982). Digestive enzymes of the salivary glands and gut of *Mastotermes darwiniensis*. *Insect Biochemistry*, **12,** 35–40.

Veivers, P. C., O'Brien, R. W. & Slaytor, M. (1980). The redox state of the gut of termites. *Journal of Insect Physiology*, **26,** 75–7.

Veivers, P. C., O'Brien, R. W. & Slaytor, M. (1982). Role of bacteria in maintaining the redox potential in the hindgut of termites and preventing entry of foreign bacteria. *Journal of Insect Physiology*, **28,** 947–51.

Waterhouse, D. F., Hackman, R. H. & McKellar, J. W. (1961). An investigation of chitinase activity in cockroach and termite extracts. *Journal of Insect Physiology*, **6,** 96–112.

Wolfe, R. S. (1979). Methanogenesis. In *International Review of Biochemistry, Microbial Biochemistry*, vol. 21, ed. J. R. Quayle, pp. 270–300. Baltimore: University Park Press.

Wolin, M. J. (1974). Metabolic interactions among intestinal microorganisms. In *Intestinal Microecology*, ed. M. H. Floch & D. J. Hentges, *American Journal of Clinical Nutrition*, **27,** 1320–8.

Wood, T. G. (1978). Food and feeding habits of termites. In *Production Ecology of Ants and Termites*, ed. M. V. Brian, pp. 55–80. Cambridge University Press.

Wood, T. G. & Sands, W. A. (1978). The role of termites in ecosystems. In *Production Ecology of Ants and Termites*, ed. M. V. Brian, pp. 245–92. Cambridge University Press.

Wood, T. M. & McCrae, S. I. (1979). Synergism between enzymes involved in the solubilization of native cellulose. In *Hydrolysis of Cellulose: Mechanisms of Enzymatic and Acid Catalysis*, ed. R. D. Brown & L. Jurasick, pp. 181–209. Washington: American Chemical Society.

Yamaoka, I. & Nagatani, Y. (1975). Cellulose digestion system in the termite, *Reticulitermes speratus* (Kolbe). I. Producing sites and physiological significance of two kinds of cellulase in the worker. *Zoological Magazine*, **84,** 23–9.

Yamin, M. A. (1978). Axenic cultivation of the cellulolytic flagellate *Trichomitopsis termopsidis* (Cleveland) from the termite *Zootermopsis*. *Journal of Protozoology*, **25,** 535–8.

Yamin, M. A. (1979). Flagellates of the orders Trichomonadida Kirby, Oxymonadida Grassé, and Hypermastigida Grassi and Foà reported from lower termites (Isoptera families Mastotermitidae, Kalotermitidae, Hodotermitidae, Termopsidae, Rhinotermitidae, and Serritermitidae) and from the wood-feeding roach *Cryptocercus* (Dictyoptera: Cryptocercidae). *Sociobiology*, **4,** 1–120.

Yamin, M. A. (1980). Cellulose metabolism by the termite flagellate *Trichomitopsis termopsidis*. *Applied and Environmental Microbiology*, **39,** 859–63.

Yamin, M. A. (1981). Cellulose metabolism by the flagellate *Trichonympha* from a termite is independent of endosymbiotic bacteria. *Science*, **211,** 58–9.

Yamin, M. A. & Trager, W. (1979). Cellulolytic activity of an axenically-cultivated termite flagellate, *Trichomitopsis termopsidis*. *Journal of General Microbiology*, **113,** 417–20.

Yokoe, Y. (1964). Cellulase activity in the termite, *Leucotermes speratus*, with new evidence in support of a cellulase produced by the termite itself. *Scientific Papers of the College of General Education, University of Tokyo*, **14,** 115–20.

Zimmerman, P. R., Greenberg, J. P., Wandiga, S. O. & Crutzen, P. J. (1982). Termites: a potentially large source of atmospheric methane, carbon dioxide, and molecular hydrogen. *Science*, **218,** 563–5.